American Journal of Medical Genetics 69:89–95 (1997)

Maternal Phenylketonuria Collaborative Study (MPKUCS) Offspring: Facial Anomalies, Malformations, and Early Neurological Sequelae

Bobbye Rouse,[1*] Colleen Azen,[2] Richard Koch,[2] Reuben Matalon,[3,4] William Hanley,[5] Felix de la Cruz,[6] Friedrich Trefz,[7] Eva Friedman,[2] and Harvey Shifrin[6]

[1]*Children's Hospital, University of Texas Medical Branch, Galveston*
[2]*Children's Hospital Los Angeles, Los Angeles, California*
[3]*Nutrition and Medical Dietetics, University of Illinois, Chicago*
[4]*Research Institute, Miami Children's Hospital, Miami, Florida*
[5]*Hospital for Sick Children, Toronto, Ontario, Canada*
[6]*National Institute of Child Health and Human Development, NIH, Bethesda, Maryland*
[7]*Universitats of Tuebingen Kinderklinik, Reutlingen, Germany*

Maternal phenylketonuria (PKU) in untreated women has resulted in offspring with microcephaly, mental retardation, congenital heart disease (CHD), and intrauterine growth retardation. The Maternal Phenylketonuria Collaborative Study (MPKUCS) was designed to determine the effect of dietary control of blood phenylalanine (Phe) during pregnancy in preventing damage to the fetus associated with untreated Maternal PKU. A cohort of offspring from MPKUS pregnancies was ascertained and examined to evaluate malformations, including CHD, craniofacial abnormalities, microcephaly, intrauterine and postnatal growth retardation, other major and minor defects, and early abnormal neurological signs. For analysis, the women were grouped according to their mean Phe levels in µmol/liter, ≤360, 361–600, 601–900, or >900, during critical gestational weeks of 0–8 (N = 203) and 8–12 (N = 190), and average for Phe exposure throughout pregnancy (N = 183).

Frequencies of congenital abnormalities increased with increasing maternal Phe levels. Significant relationships included average Phe 0–8 weeks and CHD ($P = 0.001$); average Phe 8–12 weeks and brain, fetal, and postnatal growth retardation ($P < 0.0005$ for all), wide nasal bridge ($P < 0.0005$), and anteverted nares ($P = 0.001$); and average Phe exposure during the entire pregnancy and neurological signs ($P < 0.0005$). Although 14% of infants had CHD, none of the CHD occurred at 120–360 µmol/liter and only one (3%) at 361–600 µmol/liter. At levels of 120–360 µmol/liter, there were three infants (6%) with microcephaly, two (4%) with postnatal growth, and none with intrauterine growth retardation, in contrast to 85%, 51%, and 26%, respectively, with Phe above 900 µmol/liter. These data support the concept that women with PKU should begin a low-phenylalanine diet to achieve Phe levels of <360 µmol/liter prior to conception and should maintain this throughout pregnancy. Am. J. Med. Genet. 69:89–95, 1997.

KEY WORDS: MPKU; maternal phenylketonuria; congenital heart disease; microcephaly; facial dysmorphology

INTRODUCTION

Phenylketonuria (PKU) is an inborn error of phenylalanine (Phe) metabolism that, if not treated neonatally, usually causes severe mental retardation. Newborn screening began 30 years ago and has resulted in a population of young PKU adults with normal intelligence who have reached childbearing age. Many of these women have discontinued their low-Phe diet and

Contract grant sponsor: National Institute of Child Health and Human Development; Contract grant numbers: NOI-HD-2-3156 (Galveston), NOI-HD-2-3155 (Chicago), NOI-HD-2-3148 (Los Angeles), NOI-HD-2-3149 (Boston); Contract grant sponsor: National Health Research and Development Program; Contract grant number: 6606-3265; Contract grant sponsor: NIH; Contract grant number: 00073.

In memory of Tony Lipson.

*Correspondence to: Bobbye Rouse, M.D., Children's Hospital, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0319.

Received 4 April 1996; Accepted 15 July 1996

now run the risk of damaging their offspring if they become pregnant without being on a Phe-restricted diet. Dent [1957] and Mabry et al. [1963] first described what has become known as maternal PKU (MPKU). In a retrospective survey, Lenke and Levy [1980] documented the increased frequency of mental retardation, low birth weight, microcephaly, congenital heart disease (CHD), and other defects in offspring of women with untreated hyperphenylalaninemia (HPA). Kirkman [1982] predicted that, if HPA women continue to reproduce at a normal rate without dietary treatment, the occurrence of mental retardation due to untreated PKU could return after only one generation to a level that preceded newborn screening.

Dietary control of Phe intake during pregnancy may be the answer to this problem; however, there is limited information concerning the timing and degree of control necessary to prevent damage to the fetus. This lack of information prompted the National Institute of Child Health and Human Development to issue in 1983 a request for proposals. The Maternal PKU Collaborative Study was initiated the following year. The study design involved a Coordinating Center and four Contributing Centers, with participation of clinics throughout the United States. Each would follow a uniform protocol. Canada joined the study in 1985 and Germany in 1991. The details of the study design were published and updated [Koch et al., 1990, 1993]. The study proposes to evaluate the efficacy of a Phe-restricted diet before and during pregnancy to reduce fetal morbidity and mortality.

The purpose of this report is to describe the phenotype of the offspring of women with HPA. Data will be reported in terms of Phe levels during critical periods of prenatal development.

METHODS

A standard protocol and manual of procedures were developed in 1984 by the principal investigators and project officers of the National Institute of Child Health and Human Development. Categories of HPA with the subject on an unrestricted diet were classified as follows: 1) Classical PKU subjects have blood Phe levels ≥1,200 μmol/liter (≥20 mg/dl), normal blood tyrosine (Tyr), and large amounts of Phe metabolites in their urine; 2) atypical PKU subjects have blood Phe levels between 600 and 1,199 μmol/liter (10–19.9 mg/dl), normal blood Tyr, and small to moderate amounts of Phe metabolites in their urine; and 3) mild (benign) HPA subjects have blood Phe levels between 240 and 599 μmol/liter (4–9.9 mg/dl), normal blood Tyr, and few or no Phe metabolites in their urine [Koch et al., 1990].

The HPA women were classified according to their natural Phe levels on a normal diet. If they were pregnant at enrollment, these levels were established after delivery of the offspring. The women with blood Phe >360 μmol/liter (6 mg/dl) on a normal diet were started on a Phe-restricted diet to maintain their blood Phe levels between 120 and 360 μmol/liter (2–6 mg/dl) prior to and throughout pregnancy. Non-PKU controls were recruited from the obstetricians' practices and from among members of the families of the subjects. The outcome measures evaluated included [Koch et al., 1993]: 1) rate of spontaneous abortions, 2) length of gestation, 3) fetal growth, 4) birth measurements, 5) malformations, 6) postnatal growth, and 7) developmental and neurological maturation of the offspring.

Gestational age of the offspring was based on date of last menstrual period, confirmed by ultrasound in 51% of subjects, on last menstrual period alone in 14% of subjects, on ultrasound alone in 26% of subjects, and on initial examination or neonatal evaluation in 9% of subjects. All methods of dating agreed within 2 weeks in 60% of births. The birth measurement centiles were adjusted for gestational age according to the Swedish norms [Niklasson et al., 1991]. The newborn infants had a complete physical examination, and, when possible, craniofacial measurements were taken by a neonatologist or developmental pediatrician and recorded on a standard worksheet. General principles for measuring body parts and for curves of normal values were available to all of the participating clinics [Goodman and Gorlin, 1983]. The worksheets were designed to record the physical examination, both descriptive and specific measurements of each area of the body. The facial measurements included the length between canthi and the length of the philtrum. The length, position, and structure of the ears and angle of the nares were recorded, as were observations regarding the palate. Copies of the physical examination forms were sent to one of the contributing centers for analysis by a person experienced in clinical genetics and malformations. The centiles were calculated using standard curves. The data were evaluated independently by a different person, a clinical geneticist, for quality control [Merlob et al., 1984; Feingold and Bossert, 1974; Goodman and Gorlin, 1983; Nellhaus, 1968]. Deviations of 2 SD or greater were considered abnormal.

When clinically indicated, the newborn infants were seen by subspecialists, such as geneticists, cardiologists, and neurologists. After the initial examination, the infants continued to be followed for their growth, physical, neurological, and developmental status. The neurological examination included newborn reflexes at birth and reflexes during the early months. Subsequent examinations included overall neurological status, gross and fine motor skills, cranial nerves, muscle strength and tone, and the presence of normal and pathological reflexes.

Outcome variables in this report were analyzed in relation to maternal Phe levels during critical periods of gestation, 0–8 weeks and 8–12 weeks, and in relation to average Phe exposure throughout the pregnancy. Women were grouped according to whether their average Phe levels during specified periods fell to ≤360 μmol/liter, 361–600 μmol/liter, 601–900 μmol/liter, or >900 μmol/liter. The numbers of women whose offspring had abnormalities on each of the outcome variables were compared across Phe categories by using the χ^2 test. Probabilities of less than 0.01 were considered statistically significant. Stepwise multiple logistic regression was used to investigate the independent effects of maternal age, smoking, and alcohol use in addition to the effects of Phe levels. Statistical analyses were performed by using SAS [SAS Institute, Inc., 1988] and BMDP [Dixon, 1990] software packages.

RESULTS

Among the 468 pregnancies in the study, 331 resulted in live births; 28% of pregnancies resulted in pregnancy terminations, 13% spontaneous and 15% elective. There were two still births and two ectopic pregnancies. Although 331 babies had been born as of July 1, 1994, the sample reported represents those whose evaluation was received in time for comprehensive quality control and analysis and those with CHD who had definitive diagnostic procedures completed. These evaluations were received for 88% of infants born before January 1, 1993. Those born before 1993 for whom there is follow-up data had higher maternal Phe exposure (558 μmol/liter vs. 300 μmol/liter; $P = 0.0001$). The additional babies born in 1993 and 1994 for whom there is follow-up data were born at an earlier gestational age (37.7 vs. 39.4 weeks; $P = 0.004$) and had mothers with lower IQs (82 vs. 91, $P = 0.005$).

Table I shows the diagnostic classification of the mothers included in this analysis. These included 134 with classical PKU (59%), 42 with atypical PKU (19%), 37 with mild HPA (16%), and 14 undetermined at the time of the analysis (6%). All but two of the mild HPA cases were untreated, whereas only one atypical and three classic PKU (2% each) patients declined treatment.

Tables II and III show the weeks gestation at diet initiation and number of weeks after diet initiation before control was achieved. The gestational age at initiation of treatment was similar for the atypical and classical groups (median 5.4 and 6.4 weeks, respectively), but the classical PKU mothers took longer to achieve Phe levels consistently below 600 μmol/liter (median 12 weeks vs. 1 week for the atypical group). Nevertheless, among the classical PKU mothers, 20 had average Phe levels below 360 μmol/liter during the period covering weeks 8–12 of gestation, and 21 had average Phe levels between 360 and 600 μmol/liter. Of the live births, 203 had blood Phe level measurements during weeks 0–8 of gestation that were used for data analysis for CHD. One hundred ninety had Phe level measurements for weeks 8–12 of gestation. One hundred eighty-eight had serial growth data, including weight, length, and head circumference from birth through age 7 years. These data were used for analysis of brain, fetal, and body growth. Facial findings were analyzed for 186 of the offspring. Average Phe exposure and complete neurological data were available for analysis on 183 babies.

Facial Findings

Facial development occurs chiefly between the third and eighth weeks of gestation, but the final facial proportion develops between the tenth and fourteenth gestational weeks [Moore, 1973; Sadler, 1985]. The craniofacial abnormalities were as follows: wide flat nasal bridge, wide outer canthus, long palpebral fissures, long and/or smooth philtrum, and anteverted nares. Epicanthal folds and high arched palate were noted in infants. Ear abnormalities included apparently low-set, posteriorly angulated, poorly developed auricles and/or large ears. It was anticipated that these signs would have the strongest relationship to the blood Phe levels during weeks 8–12. As is shown in Figures 1 and 2, there is a progressive increase in the percentage of several of the abnormalities as the Phe level rises above 900 μmol/liter. Facial structure was abnormal even at well-controlled levels in 50% or more of our cohort, except for the abnormalities of the nares, palate, and epicanthal folds. Because morphogenesis begins at 3–4 weeks of gestation and many of these women had elevated levels in that earlier period, this could explain the higher percentages of abnormalities for the 8–12 week period. For two of the anomalies, wide flat nasal bridge and anteverted nares, the relationship is significant ($P < 0.0005$ and $P = 0.001$, respectively). In addition, the higher the Phe levels the greater were the number of minor anomalies in any one infant.

TABLE I. Diagnostic Classification of Mothers

Type	n	%	Not treated
Classical PKU	134	59	3
Atypical PKU	42	19	1
Mild HPA	37	16	35
Unknown	14	6	
Total	227	100	

TABLE II. Weeks of Gestation at Diet Initiation

	Atypical PKU		Classical PKU	
	5.4 weeks		6.4 weeks	
Median	n	%	n	%
Preconception	12	29	30	22
0–10 weeks	19	45	70	52
>10 weeks	10	24	31	23
Not treated	1	2	3	2
Total	42		134	

Growth and Neurological Abnormalities

Whereas the critical period in gestation for the development of organs is during the period of rapid cell division or differentiation, the most important period for brain growth is between weeks 3 and 16. Differentiation extends into infancy, whereas body growth extends beyond that time [Sadler, 1985]. The data on brain growth and intrauterine and postnatal growth were analyzed by using blood Phe levels for gestational periods 0–8 weeks and 8–12 weeks, and average Phe exposure during the entire pregnancy.

TABLE III. Number of Weeks After Diet Initiation to Achieve Control

	Atypical PKU		Classical PKU	
	1.0 weeks		12.0 weeks	
Median	n	%	n	%
Within 2 weeks	17	59	30	30
2–20 weeks	10	34	31	31
20–34 weeks	0		8	8
Never	2	7	32	32
Total	29		101	
Pre-conception Rx[a]	12		32	

[a] Could not determine number of weeks to achieve control due to insufficient preconception data.

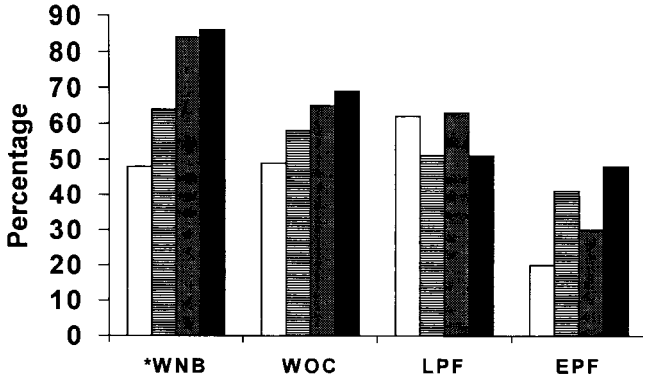

Fig. 1. Relationship between minor anomalies and blood Phe (μmol/liter) during gestational weeks 8–12. WNB, wide nasal bridge; WOC, wide outer canthus; LPF, long palpebral fissures; EPF, epicanthal fold. For WNB, $P < 0.0005$. □ 120–360 (n = 48); ▤ 361–600 (n = 41); ▩ 601–900 (n = 55); ■ > 900 (n = 42).

Brain, fetal, and body growth related to Phe levels during weeks 8–12 of gestation were all significant, at $P < 0.0005$. One hundred eighty eight women had offspring with complete growth data. Of those who had good control i.e., Phe levels between 120 and 360 μmol, only three offspring or 6% had microcephaly, none had intrauterine growth retardation, and only 4% had postnatal growth retardation. At mean Phe levels between 361 and 600 μmol, 15% had microcephaly, 2% had intrauterine growth retardation, and 22% had postnatal growth retardation. Figure 3 shows this relationship and the increasing frequency of abnormalities as the blood Phe rises to 900 μmol/liter and higher.

The nervous system begins embryogenesis during the third week and continues throughout the sixteenth week. Further differentiation continues, as does myelinization, through the first year of life [Moore, 1988]. One hundred eighty-three women had offspring with complete neurological data. Of the 52 who were in

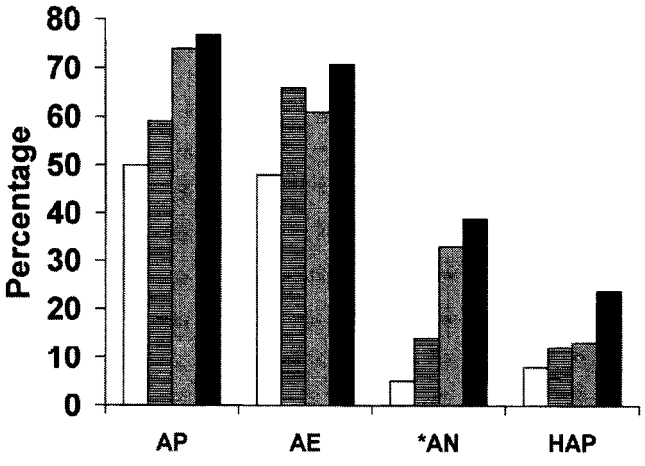

Fig. 2. Relationship between minor anomalies and blood Phe (μmol/liter) during gestational weeks 8–12. AP, abnormal philtrum (smooth or long); AE, abnormal ears (large, low set, posterior angulation, auricle defect); AN, anteverted nares; HAP, high arched palate. For AN, $P = 0.001$. □ 120–360 (n = 48); ▤ 361–600 (n = 41); ▩ 601–900 (n = 55); ■ > 900 (n = 42).

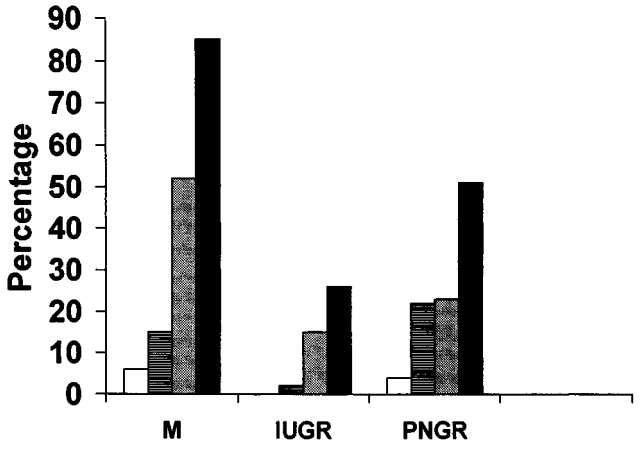

Fig. 3. Relationship between brain, fetal, and body growth and blood Phe (μmol/liter) during gestational weeks 8–12. M, microcephaly; IUGR, intrauterine growth retardation; PNGR, postnatal growth retardation. $P < 0.005$. □ 120–360 (n = 48); ▤ 361–600 (n = 41); ▩ 601–900 (n = 52); ■ > 900 (n = 47).

good control (between 120 and 360 μmol/liter) only two, or 4%, of the offspring, had general neurological abnormalities. The others had abnormalities, including abnormal tone (hypertonia, hypotonia), fine and/or gross motor delay, and abnormal reflexes (hyperreflexia, persisting primitive reflexes). Fourteen percent of those with Phe levels between 361 and 600 μmol/liter had motor delay, 13% had abnormal tone, and 11% had abnormal reflexes. Figure 4 shows the increased frequency of neurological abnormalities with the increase in average Phe exposure, throughout the entire pregnancy. This relationship was significant at $P < 0.0005$.

Congenital Defects of the Heart and Great Vessels

The critical period of cardiogenesis is between days 20 and 50, or the third through the eighth week of gestation [Nora and Nora, 1978; Moore, 1988]. Obviously, dynamic changes continue throughout the pregnancy as the original cardiac tube goes through differentiation into a

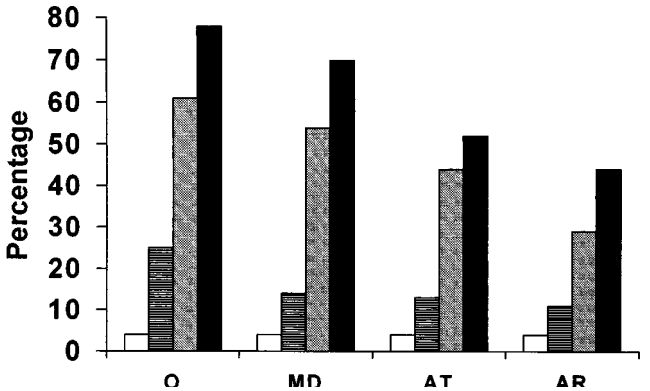

Fig. 4. Relationship between neurological abnormalities and average Phe exposure (μmol/liter). O, overall; MD, motor delay; AT, abnormal tone; AR, abnormal reflexes. $P < 0.0005$. □ 120–360 (n = 52); ▤ 361–600 (n = 63); ▩ 601–900 (n = 41); ■ > 900 (n = 27).

TABLE IV. Relationship of Congenital Heart Defects to Blood Phe (μmols/L) During 0–8 Weeks Gestation*

Phe	n	120–360 (n = 33)	361–600 (n = 34)	601–900 (n = 37)	>900 (n = 99)
Tetralogy of Fallot[a]	4				4
Coarctation	7			1	6
Single	(2)				(2)
Multiple	(5)			(1)	(4)
Hypoplastic L.H.	2				2
P.D.A.	7		1	2	4
Single	(5)		(1)	(1)	(3)
Multiple	(2)				(2)

* Hypoplastic L.H. = Hypoplastic left heart; PDA = Patent ductus arteriosus.
$P = 0.001$.
[a] Two died.

four-chambered organ. The great vessels are forming during this same critical period, between weeks 3 and 8.

Tables IV and V show the relationship between CHD and Phe levels during gestation weeks 0–8. Twenty-eight children (14%), out of 204 infants, had CHD. Six of these infants died, two with tetralogy of Fallot, two with hypoplastic left heart syndrome, one with truncus arteriosus Type I, and one with a severe arrhythmia (no autopsy). All the mothers of children with CHD had mean Phe levels greater than 600 μmol/liter during the first 8 weeks of gestation, except one whose level was between 361 and 600 μmol/liter and who had an infant with a patent ductus arteriosus. That relationship was significant, at $P = 0.001$. However, when Phe levels during weeks 8–12 of gestation were analyzed, four mothers whose Phe levels were between 361 and 600 μmol/liter had infants with CHD.

The multiple defects seen were as follows: 1) coarctation, with a) mild aortic stenosis; b) small left ventricle, small aortic root, and 13 ribs; c) membranous ventricular septal defect, patent ductus arteriosus, right ventricular hypertrophy, and mitral stenosis; d) ventricular septal defect and subaortic stenosis; and e) atrial septal defect and patent ductus arteriosus; 2) patent ductus arteriosus with a) a ventricular septal defect and b) a large patent foramen ovale; and 3) atrial septal defect with a) right ventricular hypertrophy and b) a membranous ventricular septal defect and a large patent foramen ovale.

Other Congenital Abnormalities

Table VI shows a list of occasional congenital abnormalities. There were 65 children with one abnormality and 36 with two or more abnormalities. One infant with esophageal atresia died of sepsis at 3 months of age.

Multiple logistic regression analyses demonstrated that maternal Phe level was the most significant predictor of abnormal outcome. Alcohol use, as self-reported by these women, showed no additional impact on any of the outcome variables. After controlling for the maternal Phe, mothers under 20 years of age were more likely to have infants with neurological abnormalities (odds ratio 4.4, 95% confidence interval 1.37–14.2) than those over age 20 years, as were those who reported smoking immediately before or during pregnancy (odds ratio 3.7, 95% confidence interval 1.02–13.2). Offspring of smokers were also more likely to experience postnatal growth retardation (odds ratio 2.2, 95% confidence interval 1.2–6.5) after controlling for maternal Phe levels during pregnancy.

DISCUSSION

Congenital malformations are known to result from genetic or environmental influences or a combination of these, called multifactorial determination. Malformations may be single or multiple and may be severe or mild. Although minor anomalies are sometimes of no clinical significance, their presence should alert the clinician to look closely for major anomalies. The effect

TABLE V. Relationship of Congenital Heart Defects to Blood Phe (μmols/L) During 0–8 Weeks Gestation*

Phe	n	120–360 (n = 33)	361–600 (n = 34)	601–900 (n = 37)	>900 (n = 99)
A.S.D.	3				3
Single	(1)				(1)
Multiple	(2)				(2)
V.S.D.	2				2
Other:	3			1	2
Truncus[a]	(1)			(1)	
Harsh murmur	(1)				(1)
Arrhythmia[a]	(1)				(1)

* ASD = Atrial septal defect; VSD = Ventricular septal defect.
$P = 0.001$.
[a] Died.

TABLE VI. Occasional Congenital Abnormalities

Hair	Sparse, unruly, multiple whorls
Skin	Hemangioma, verruca, cafe au lait spots
Neck	Short, full, slightly webbed
Chest	Wide, shield-like
Hernia	Hiatal, umbilical, inguinal
Genitalia	Undescended testes, hypospadius, small testes, hydrocele, abnormal clitoris
Sacrum	Pilonidal pits and deep dimples
Extremities	Abnormal hips, pes cavus, club foot, abnormal acromion, postaxial polydactyly, clinodactyly (fingers, toes), hypoplastic nails and finger tips and simian lines
Major organs	Esophageal atresia, duplication of a kidney, pulmonary hypoplasia with lobar emphysema
Other minor	Ptosis, abnormal uvula, micrognathia, short palpebral fissures

of any teratogen is thought to be closely related to the timing and degree of exposure during critical periods of embryogenesis. Although specific periods of gestation may reflect the most sensitive time for a particular system, structural and functional development may extend well beyond the critical period. The effects on some systems would be expected to accrue throughout the pregnancy.

Stevenson and Huntley [1967] reported the earliest comprehensive data on offspring of two women with PKU. These women had, between them, 16 spontaneous abortions (62% of their pregnancies) and ten live births. Six of their ten babies had confirmed congenital heart defects, the most common being coarctation and patent ductus arteriosus. Stevenson and Huntley's conclusion was that the "maternal PKU embryopathy syndrome" includes microcephaly, low birth weight, postnatal growth retardation, mental retardation, congenital hip dislocation, and congenital heart disease. The lack of blood Phe monitoring precluded an association with PKU as a potential teratogen.

Lipson et al. [1981, 1984] noted a pattern of malformations, including mental retardation, growth deficiency, central nervous system dysfunction, severe and mild malformations, and minor facial anomalies. Using the data from Lenke and Levy [1980] and from Müller et al. [1979], and their own data, Lipson et al. [1984], showed a relationship between an increasing frequency of defects and rising blood Phe levels. These reports reflect retrospective data. In 1984, Lipson et al. reported on 34 children of 11 untreated hyperphenylalaninemic women. Eleven of twenty-four children had reliable birth records and all had intrauterine growth retardation, and 22 of 24 with growth records had postnatal growth retardation. All 34 children had microcephaly, 20% had strabismus, 23% had seizures, and two of 34 had mild motor spasticity. Minor facial anomalies included undeveloped, long philtrum, thin upper lip, flat nasal bridge, epicanthal folds, small upturned nose, maxillary hypoplasia, and micrognathia. Seventeen percent had congenital heart defects, the most frequent being patent ductus arteriosus, ventricular or atrial septal defects, or a combination of these.

Rouse et al. [1990], using prospective data, reported an increased frequency of microcephaly, intrauterine growth retardation, minor facial anomalies, and congenital heart defects in offspring of HPA women whose average blood Phe during pregnancy exceeded 600 μmol/liter. Bachman et al. [1993] reported on a woman who was discovered to have PKU following the birth of two children with typical minor facial anomalies, mental retardation, and microcephaly. She was started on a diet and had Phe levels just below 600 μmol/liter at her next conception. During the remainder of the pregnancy, she had levels between 360 and 600 μmol/liter, except for the fifth and sixth weeks, when the levels of Phe were 600–720 μmol/liter. Her third infant had minor facial anomalies and delayed development.

In most reports, the affected infants have a similar facial appearance; however, they do not all look alike. In our cohort, 15 infants had only one minor facial anomaly and 50 had five or more. With the blood Phe of less than 360 μmol/liter during gestational weeks 8–12, the frequency of midfacial abnormalities, of the philtrum and ears, was unexpectedly high in our cohort. The fact that the mean blood Phe was greater than 360 μmol/liter during the first 8 weeks in many of these women might explain the high frequency of midfacial and ear abnormalities. Facial development, derived from the frontonasal prominence of the embryo, begins early in the third gestational week, which is an exceptionally sensitive time for a teratogenic effect to occur. The mothers of ten of the 24 offspring with midface, philtrum, nares, and ear abnormalities had mean Phe levels ranging from 361 to 900 μmol/liter during the period from conception to week 8 of pregnancy. It is important to note that only three of 48 offspring in the $<$360 μmol/liter group had microcephaly; none had intrauterine growth retardation, and two had postnatal growth retardation. Only two of 52 had overall neurological abnormalities. Equally important is that the MPKU women who had offspring with congenital heart and great vessel abnormalities had mean Phe levels $>$601 μmol/liter during weeks 0–8 of gestation, except for one mother whose child had a patent ductus arteriosus that required surgical closure. As blood Phe rises, the abnormalities increase in frequency at several critical periods of embryogenesis and organogenesis. The data clearly indicate that blood Phe is teratogenic in the infants. The results reported support this assumption, as did the obstetric findings of Platt et al. [1992]. There is no question that not only should women with phenylketonuria be started on low-phenylalanine dietary therapy before conception, but blood Phe must also remain within the recommended range of 120–360 μmol/liter throughout pregnancy.

Lipson et al. [1981, 1984] noted that the pattern of malformations and some facial anomalies of offspring of women with PKU are similar to those reported in fetal alcohol syndrome. Furthermore, the authors indicated that severity of facial anomalies depends on several factors: the degree and chronicity of the agent, the genotype of the parent and fetus, and the timing of the exposure of the embryo to the teratogen. The current report agrees with this theory except for the conclusion regarding genotype. More data on the DNA of our cohort are required to answer that question. DNA of the women and offspring is being collected for analysis.

Kirby and Miyagawa [1990] used chick embryo to show the effect of high phenylalanine on embryonic development. After having treated the embryos with phenylalanine in the first few days, they demonstrated several serious heart defects in the chicks. The exact mechanism remains unclear, although disruption of neural crest cell migration appears to have a role. The neural crest is said to contribute to the development of many organs, including the heart, aortic arch derivatives, and face, all of which are affected in maternal phenylketonuria.

A mutant mouse model has been developed that biochemically closely simulates the human with PKU [McDonald et al., 1990]. This animal has been used to study gene therapy, and early studies are in progress to gain knowledge on congenital malformations of the heart and great vessels and the brain. Preliminary analysis shows defects of brain myelination [Dyer et al., 1995].

ACKNOWLEDGMENTS

We express our appreciation to all of the professionals who contributed patients and data to this study and to the families who participated. This research was supported by the National Institute of Child Health and Human Development contracts NOI-HD-2-3156 (Galveston), NOI-HD-2-3155 (Chicago), NOI-HD-2-3148 (Los Angeles), and NOI-HD-2-3149 (Boston) and by the National Health Research and Development Program, Canada, project 6606-3265, and General Clinical Research Center, Galveston, Texas (NIH grant 00073).

REFERENCES

Bachman R, Schoen E, Backstrom M, Lee R, Williams S, Jurecki E (1993): Phenylalanine embryopathy in three siblings: implications of maternal diet therapy. The pediatric forum. Am J Dis Child 147:22–23.

Dent CE (1957): Relation of biochemical abnormality to development of mental defect in phenylketonuria. In: "Report of 23rd Ross Pediatric Research Conference." Columbus, OH: Ross Laboratories, p 32.

Dixon WJ (ed; 1990): "BMDP Statistical Software Manual," Vol 2. Berkeley, CA: University of California Press.

Dyer C, Philibotte T, Levy H, Cruz J (1995): Mechanisms of hypomyelination and gliosis in a genetic mouse model for phenylketonuria. J Neurochem 64(Suppl):S111.

Feingold M, Bossert WH (1974): Normal values for selected physical parameters: An aid to syndrome delineation. Birth Defects: Orig Art Ser 10(13).

Goodman R, Gorlin R (1983): "The Malformed Infant and Child." New York: Oxford University Press.

Kirby M, Miyagawa S (1990): The effects of high phenylalanine concentration on chick embryonic development. J Inher Metab Dis 13:634–640.

Kirkman HN (1982): Projections of a rebound in frequency of mental retardation from phenylketonuria. Appl Res Ment Retard 3: 319–328.

Koch R, Hanley W, Levy H, Matalon R, Rouse B, de la Cruz F, Azen C (1990): A preliminary report of the collaborative study of maternal phenylketonuria in the United States and Canada. J Inher Metab Dis 13:641–650.

Koch R, Levy H, Matalon R, Rouse B, Hanley W, Friedman E, Azen C, de la Cruz F (1993): The North American Collaborative Study of Maternal Phenylketonuria (PKU). Int Pediatr 8:89–96.

Lenke RR, Levy HL (1980): Maternal phenylketonuria and hyperphenylalaninemia: An international survey of the outcome of untreated and treated pregnancies. N Engl J Med 303:1202–1208.

Lipson AH, Yu JS, O'Halloran M, Williams R (1981): Alcohol and phenylketonuria. Lancet 1:717.

Lipson AH, Buehler B, Bartley J, Walsh D, Yu J, O'Halloran M, Webster W (1984): Maternal hyperphenylalaninemia fetal effects. J Pediatr 104:216–220.

Mabry CC, Denniston JC, Nelson TL, Choon DS (1963): Maternal phenylketonuria. A cause of mental retardation in children without metabolic defect. N Engl J Med 269:1404–1408.

McDonald D, Bode V, Dove W, Shedlovsky A (1990): Pah hph-5: A mouse mutant deficient in phenylalanine hydroxylase. Proc Natl Acad Sci USA 87:1965–1967.

Merlob P, Sivan Y, Reisner S (1984): Anthropometric measurements of the newborn infant. Birth Defects: Orig Art Ser 20(7):9,11,16,22.

Moore K (1973): The Face. In: "The Developing Human," 1st Ed. Philadelphia: W.B. Saunders Co., First Edition, p. 148.

Moore K (1988): "The Developing Human," 4th Ed. Philadelphia: W.B. Saunders Co., pp 286, 373.

Müller WD, Haidvogel M, Scheibenreiter S (1979): Phenylalanin- fetopathie bei Zwillingen einer nicht diagnostizierten phenylketonurischen Mutter. Klin Pädidiatr 191:609.

Nellhaus G (1968): Head circumference from birth to eighteen years: Practical composite international and interracial graphs. Pediatrics 41:106.

Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P (1991): An update of the Swedish Reference Standards for weight, length, and head circumference at birth for given gestational age (1977–1981). Acta Paediatr Scand 80:756–762.

Nora J, Nora A (1978): Presumed vulnerable period for teratogenic influence on cardiovascular development. Genetics and counseling in cardiovascular diseases. Springfield, IL: Charles C. Thomas, pp 131, 141.

Platt L, Koch R, Azen C, Hanley W, Levy H, Matalon R, Rouse B, de la Cruz F, Walla C (1992): Maternal phenylketonuria collaborative study, obstetric aspects and outcome: The first 6 years. Am J Obstet Gynecol 166:1150–1162.

Rouse B, Lockhart L, Matalon R, Azen C, Koch R, Hanley W, Levy H, de la Cruz F, Friedman E (1990): Maternal phenylketonuria pregnancy outcome: A preliminary report of facial dysmorphology and major malformations. J Inher Metab Dis 13:289–291.

SAS Institute, Inc. (1988): "SAS/STAT User's Guide," Release 6.03. Cary, NC: SA Institute, Inc., pp 1028.

Sadler T (1985): Head and Neck In: "Langman's Medical Embryology," 5th Ed. Baltimore: Williams and Wilkins, pp 295–298, 334–343.

Stevenson R, Huntley C (1967): Congenital malformations in offspring of phenylketonuric mothers. Pediatrics 40:33–45.